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TESTS AND REFINEMENTS OF A GENERAL STRUCTURE–ACTIVITY MODEL FOR AVIAN REPELLENTS

LARRY CLARK[1,2,*] and PANKAJ SHAH[2,3]

[1]*U.S. Department of Agriculture, Animal and Plant Health Inspection Service*
Animal Damage Control, Denver Wildlife Research Center
Denver, Colorado 80225

[2]*Monell Chemical Senses Center*
3500 Market Street, Philadelphia, Pennsylvania 19104

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Abstract—We tested the robustness of a structure–activity model for avian trigeminal chemoirritants. Fourteen benzoates and acetophenones were tested using European starlings *Sturnus vulgaris* as a bioassay. In general, the previously proposed model was a reasonable predictor of repellency (i.e., irritant potency). We found that the presence of a phenyl ring was critical to repellency. Basicity of the molecule is the next most critical feature influencing repellency. The presence of an acidic function within the electron-withdrawing functionality seriously detracts from repellency. The presence or absence of an electron-withdrawing or -donating group may potentiate repellent effects, but its presence is not critical, so long as the phenyl ring is electron rich. Our data suggest that there is an *o*-aminoacetophenone/methyl anthranilate trigeminal chemoreceptor in birds analogous to the mammalian capsaicin receptor. Both receptors contain a benzene site. However, birds seem to lack the associated thiol/hydrogen-bonding site present in mammals which is needed to activate the benzene site. Rather, birds may possess an associated exposed charged site that in turn may interact with the stimulus to activate the benzene site. These differences may explain the differential sensitivity of birds and mammals to aromatic irritants.

Key Words—Acetophenones, benzoates, bird repellents, irritant, receptor model, structure–activity relationships, *Sturnus vulgaris*, trigeminal.

*To whom correspondence should be addressed.
[3]Present address, Pfizer, Inc., Box 88, Terre Haute, Indiana 47808.

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INTRODUCTION

Many birds avoid edibles based upon chemosensory cues (Schuler, 1983; Jakubas et al., 1992; Mason et al., 1989). When avoidance is nonlearned and resistant to habituation, the bird is most likely responding to a chemical irritant (Clark and Mason, 1993). Mediation of irritation is via the chemically sensitive fibers (A-delta and C fibers) of the trigeminal nerve (Finger et al., 1990). These fibers are typically found in the highest densities around mucous membranes; for birds, this corresponds to the eyes and buccal and nasal cavities.

There is evidence that irritants are perceived similarly within a vertebrate class, but there are dramatic differences in perception between classes. Birds do not avoid familiar mammalian irritants such as ammonia, gingerol, zingerone, hydroquinones, and naphthalene (Dolbeer et al., 1989; Mason and Otis, 1990). Other mammalian irritants, such as piperine, allyl isothiocyanate and mercaptobenzoic acid, have some repellent effects, but only at high concentrations ($>10{,}000$ ppm) under specific presentation schemes (Mason and Otis, 1990). The most striking example of the difference between birds and mammals is for capsaicin. Mammals uniformly avoid capsaicin (the hotness in red chilies) at about 100–1000 ppm. Birds will readily consume up to 20,000 ppm (Solzcsanyi et al., 1986; Mason et al., 1991). Yet birds avoid methyl anthranilate (grape flavoring) at 1000 ppm (Mason et al., 1989), while mammals are indifferent or prefer the compound at concentrations up to 10,000 ppm (Furia and Bellanca, 1975).

Very little is known about comparative quantitative structure–activity relationships of trigeminal irritants among the taxa. The apparent differences between birds and mammals in response to irritants suggests that avian and mammalian trigeminal receptors may not share common structures (Solzcsanyi et al., 1986). Perception of chemical pain is hypothesized to be adaptive because it allows an animal to avoid potentially dangerous compounds. The existence of differences in perception between birds and mammals raises the question about the possible selective pressures extant at the beginning of each taxonomic line.

In an effort to better understand the potentiating factors responsible for differences in chemethesis between birds and mammals, we set out to expand upon and quantify the chemical structure–behavioral activity relationships of candidate trigeminal irritants for each class. This paper focuses on refinements and tests of a previously elucidated avian model (viz., Clark and Shah, 1991; Clark et al., 1991; Shah et al., 1991). The compounds tested herein were selected to test the robustness of our model.

METHODS AND MATERIALS

Study Subjects. Adult European starlings (*Sturnus vulgaris*) were captured at the Philadelphia Zoo using funnel traps and transported to the Monell Center via car. Starlings were individually caged ($61 \times 36 \times 41$ cm) under a 12:12

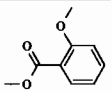
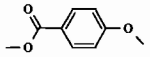
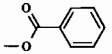
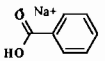
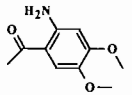
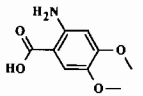
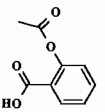
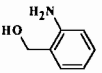
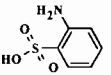
hr light–dark cycle for a two-week adaptation period and given free access to Purina Flight Bird Conditioner (Purina Mills, St. Louis, Missouri), water and oyster shell grit (United Volunteer Aviaries, Nashville, Tennessee). Starlings were chosen as test animals because previous experiments showed them to be good models of avian sensitivity (Clark and Smeraski, 1990; Clark and Shah, 1991).

Stimuli. We selected 14 chemicals on the basis of conformational, physical, and electronic attributes that could be used to test the repellency model previously generated (Figure 1). We attempted to achieve a maximum test concentration of 5000 mg/liter (0.5%); however, differences in water solubility precluded achieving this goal in many cases. Solubility data were obtained from the published literature or empirically. In all cases, concentrations were validated using UV spectrographic or HPLC methodology. Lower concentrations tested were obtained via serial dilution of the maximum concentration used. The rationale for stimulus selection is as follows.

Effects of Substituent Position. We previously found substituent position to be an important factor influencing repellency (Clark and Shah, 1991). Position of substituents affects the distribution of surface charges of the molecule, which in turn may be important for stimulus access to receptors. Groups such as $-NH_2$ and OH (and their derivatives, e.g., $-OCH_3$) act as strong activators toward electrophilic aromatic substitution, releasing their electrons via resonance rather than induction. The carbonium ion formed during electrophilic attack ortho or para to the substituent is stabilized by electron-donating groups (EDG). Furthermore when in the ortho position, the free NH_2/OH groups have a possibility of hydrogen bond interactions as in the previously tested methyl anthranilate and *o*-aminoacetophenone (Clark and Shah, 1991; Clark et al., 1991). We selected methyl 2- and methyl 4-methoxybenzoate to test the importance of substituent position in the absence of hydrogen bonding.

Effects of Substituent Position and Electron Richness of Phenyl Ring. If electron richness of the phenyl ring was the only critical feature for repellency (viz., Clark et al., 1991), then loading the ring with EDGs might be expected to enhance repellency. Previous experiments demonstrated that *o*-aminoacetophenone was a strong repellent (Clark and Shah, 1991). We selected 2-amino-4,5-dimethoxyacetophenone because it contained three EDGs. Anthranilic acid also was shown to be repellent, but less so because of its free carboxylic acid group (Clark et al., 1991). We selected 2-amino-4,5-dimethoxybenzoic acid to determine if repellency could be improved upon by loading the phenyl ring with EDGs.

Effects of Removing EDG and Altering EWG. Donation of lone pairs of electrons to the phenyl ring is associated with repellency (Clark and Shah, 1991). We selected methyl benzoate because we anticipated that the absence of an EDG on this molecule would result in a diminished repellency relative to the previ-

Code	Name	CAS	Structure
M2MOB (99%)	methyl 2-methoxybenzoate	606-45-1	
M4MOB	methyl 4-methoxybenzoate	121-98-2	
MB	methyl benzoate	93-58-3	
SB	sodium benzoate	532-32-1	
2A45DMAP	2-amino-4,5-dimethoxyacetophenone	4101-30-8	
AP	acetophenone	98-86-2	
2A45DMBA	2-amino-4,5-dimethoxybenzoic acid	5653-40-7	
ASA	acetylsalicylic acid	50-78-2	
ANTH	anthranil	271-58-9	
2ABAL	2-aminobenzyl alcohol	5344-90-1	
NNDMAN	*N,N*-dimethylanaline	121-69-7	
SAAM	2-aminobenzene sulfonic acid	88-21-1	
BALN	beta-alanine	107-95-9	
MEBALN	methyl ester of beta-alanine	2491-20-5	

All compounds were obtained from Aldrich, Milwaukee, Wisconson, USA and were of 99% + purity.

FIG. 1. Chemical structures, Chemical Abstract Service registry number (CAS), compound source, purity, and codes for stimuli.

ously tested methyl anthranilate. Acetophenone was chosen for similar reasons for comparison with the previously tested *o*-aminoacetophenone.

Sodium benzoate was selected because it did not have an EDG or the acidic proton of CO_2H, but it retained the carboxylate in anionic form, and, here, the negative charge is on the oxygen, not on the phenyl ring. Anthranil was selected because it possessed an electron-withdrawing group (EWG) but did not contain any substituent contributing to acidity due to the EWG.

We tested acetylsalicylic acid (aspirin) because it lacked an EDG, but in addition, substituents in the ortho position offered steric hinderance.

Effects of Removing EWG. 2-Aminobenzyl alcohol was selected because it represented a molecule with a strong basic EDG, but lacking an EWG. Thus, we anticipated that it would be a potent repellent as was found for veratryl alcohol (Shah et al., 1991). *N,N*-Dimethylaniline was selected for similar reasons, and for direct comparison to the previously tested dimethyl anthranilate (DMA) (Clark et al., 1991). If *N,N*-dimethylaniline proved to be a better repellent than dimethyl anthranilate, this would support the notion that an EWG is a detractor to repellency and that resonance per se is not critical to repellency. 2-Amino sulfonic acid was selected because of its stronger EWG, the effects of hetero atom(s), and also because it possesses acidity without a CO_2H group (relative to anthranilic acid).

Effects of Phenyl Ring. We selected β-alanine because its functionalities were similar to the previously tested anthranilic acid, but devoid of the phenyl ring. The methyl ester of β-alanine was selected because of similarity of functionalities to methyl anthranilate. If these functionalities are important per se, rather than the presence of a phenyl ring, then repellency should not be affected relative to the previously tested aminobenzoic acids.

Behavioral Assays. After adaptation, tap-water consumption was measured for 6 hr on each of three pretreatment days. Starlings were ranked according to mean water consumption and assigned to one of the six groups. The bird with the highest water consumption was assigned to the first treatment group, the bird with the second highest consumption was assigned to the second treatment group, and so forth to the final group, followed by a series of assignments from the final group back to group 1. Equality for water consumption among groups was validated using a one-way analysis of variance and was a prerequisite for further testing. A total of 36 birds was used for each experiment, with six birds per treatment group. Groups were randomly assigned to receive a specific concentration of a chemical during the treatment period.

After assignment to a concentration group, a series of no choice (one-bottle) tests was initiated. On the first day (pretreatment period), beginning at 0930 hr, consumption of tap water was recorded every 2 hr for the next 6 hr. On the second day, beginning at 0930 hr, birds were given their preassigned concentration of the chemical being tested. Consumption was recorded every 2 hr for

6 hr. After the test, birds were given free access to tap water. On the third day, beginning at 0930 hr, posttreatment water consumption of tap water was recorded every 2 hr for a total of 6 hr. In all cases, water was presented in 120-ml graduated Richter tubes. Starlings were visually isolated from one another as well as from the contents of the drinking tubes via opaque acrylic partitions.

So that comparisons of dose–response curves could be made from one experiment to another, consumption on the treatment and posttreatment days was standardized relative to pretreatment water intake. Experience indicated that absolute consumption varied seasonally, even after a two-week adaptation period. The ratio of treatment to pretreatment consumption was a measure of avoidance, while the ratio of posttreatment to pretreatment consumption was an index of carryover effects resulting from consumption of chemicals.

Characterization of Fluid Intake for Individual Compounds. A two-factor repeated measures analysis of variance was used to test whether an individual compound was repellent. The between-subjects factor was concentration group with six levels. The within-subjects (repeated) factor was period with two levels (treatment day and posttreatment day). The dependent variable was the fluid intake relative to the pretreatment fluid intake. Thus, if a chemical was repellent, its score would be less than 1.0. Duncan's multiple-range tests were used to test for post-hoc differences among means, with significance set at $P < 0.05$.

Avoidance may be attributable to sensory irritation of the trigeminal nerve and/or a postingestional conditioned aversion (Clark and Mason, 1993). Learned aversions are characterized by initial normal consumption, followed by reduced intake during subsequent observations periods. In contrast, trigeminally mediated repellency does not require learning. Trigeminal repellents cause irritation (pain) and are avoided upon initial contact. Thus, intake tends to be similar across sampling periods. Each chemical was evaluated for its mode of action using a two-way repeated measures anova. The dependent variable was absolute amount of treated water consumed. The between-subjects effect was concentration, while the within-subjects effect was hours with three levels. Only the interaction term and the main within-subjects effect were of interest. Duncan's multiple-range tests were used to determine post-hoc differences among means.

Comparison among Compounds: Operational Definitions of Sensitivity to Repellents. In most cases the dose–response relationship was best described by a nonlinear four parameter logistic equation of the form, R (response) $= (a - d)/[1 + (x/c)^b] + d$, where a is the asymptotic maximum relative consumption, b is the slope, d is the asymptotic minimum relative consumption, c is the inflection, and x is the concentration in milligrams per liter (Table 1). The minimum asymptotic consumption was constrained by a value of: mean − one standard error, in cases where the highest concentration tested was at the solubility limit of the compound. Otherwise, the minimum asymptotic value was constrained to be greater than zero, since negative intake was not a permissible

TABLE I. PARAMETER VALUES FOR DOSE–RESPONSE RELATIONSHIPS OF CANDIDATE BIRD REPELLENTS

Code[a]	*a* maximum	*b* slope	*i* inflection	*d* minimum	intercept
M2MOB	.887	1.926	2013	0.01	
M4MOB	1.088	14.704	107	0.817	
2A45DMAP	1.036	1.966	170	0.262	
2A45DMBA		−0.035			1.135
MB	0.970	6.279	554	0.613	
AP	1.005	1.030	536	0.289	
SB		−0.035			0.911
ANTH	1.001	1.571	636	0.015	
ASA	1.067	4.967	1429	0.525	
2ABAL	1.200	1.068	435	0.180	
NNDMAN	0.880	1.257	827	0.300	
2ASAD		−0.154			1.345
BALN		0.010			0.959
MEBALN	1.011	8.093	3416	0.611	
OAP[a]	0.87	11.991	204	0.010	
MA[a]	1.03	1.745	980	0.060	

[a]Reference compounds used in the laboratory (Clark and Shah, 1991; Clark et al., 1991).

value. In the remaining cases, relative intake was independent of concentration, and a log linear equation was used to characterize the relationship. Selection of models was based upon minimization of the least squared error term.

There are several ways to estimate potency of a repellent. The slope is a measure of sensitivity to changes in irritant concentration. At the extremes, large values for slope indicate an "all-or-none" threshold response, i.e., birds may be insensitive to the repellent up to a threshold concentration, after which intake is maximally suppressed. Small values for slope indicate a graded threshold response, i.e., the suppression of fluid intake is more moderate per unit change of irritant concentration. Another measure of sensitivity is the displacement of the dose–response curve along the concentration axis as estimated by the inflection point. Low inflection values (leftward shift of the curve) indicate heightened responsiveness to irritant concentrations. High inflection values (rightward shift of the curve) indicate diminished responsiveness to the irritant. The final measure of sensitivity is suppression of fluid intake as measured by the minimum asymptotic consumption. Relying on a single measure, e.g. inflection, as used in toxicological studies, to categorize irritant potency may be misleading. For example, acetophenone and methyl benzoate have relatively similar inflection points (Table 1). Using this criterion, one would conclude that these two com-

pounds have similar potency. However, inspection of the minimum asymptotic intake shows that acetophenone yields a far greater suppression of fluid intake. Thus, the question becomes: which of the available dose–response indices is the most appropriate index of potency?

To better estimate which measures of sensitivity (or combinations thereof) should be used to characterize repellent potency, we used a principal-components analysis to define descriptive dimensions. z-transformed parameter values for the nonlinear logistic dose–response curves were used as variables. Similarities of compounds based upon a dissimilarity matrix (i.e., cosine) of principal axis scores were estimated using cluster analysis. A weighted average method was used to estimate linkage among groups. The variables weighted most heavily for each significant principal component axis were operationally defined as the relevant measures of sensitivity. These measure were then used to evaluate repellent potency relative to structural features of molecules.

RESULTS

Dose–Response Relationships of Individual Compounds. Maximum suppression of intake for methyl 2-methoxybenzoate was 15% of normal levels at 4578 mg/liter. The concentration-group profiles between the treatment and posttreatment periods for methyl 2-methoxybenzoate differed ($F = 3.21$, $df = 5$, $P = 0.02$). The post-hoc test for the day of treatment showed that intake for birds receiving 4578 mg/liter was less than for any other concentration group (Figure 2A, M2MOB). Intake for all groups during the posttreatment period returned to pretreatment levels. Absolute consumption differed across concentration groups and time ($F = 2.03$, $df = 2,60$, $P = 0.046$). Birds presented with concentrations of 915 mg/liter or more showed signs of enhanced avoidance during the fourth and sixth hours of exposure, suggesting that repellency was a postingestion conditioned response. At concentrations of 476 mg/liter or less consumption was constant across hours.

Intake of methyl 4-methoxybenzoate was 85% of normal at the highest concentration tested (Figure 2B, M4MOB), although the relative fluid intake profiles for the treatment and posttreatment days differed ($F = 2.80$, $df = 5,30$, $P = 0.034$). Post-hoc analysis across concentration-groups for the treatment day indicated that birds receiving 203 mg/liter differed from those receiving 20 mg/liter. There were no other differences among concentration groups. Fluid consumption returned to pretreatment levels for all groups during the posttreatment period. Absolute consumption for each of the concentration groups was similar as a function of time, indicating that the starlings showed no evidence of learned aversions ($P = 0.446$).

Intake of 2-amino-4,5-dimethoxyacetophenone was maximally reduced to

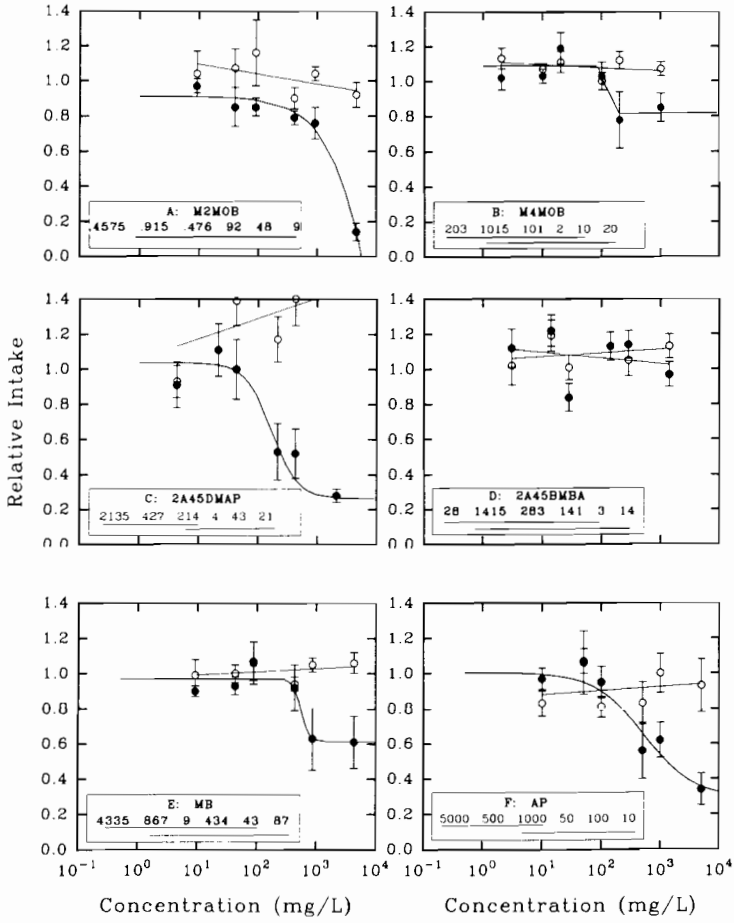


FIG. 2. Dose–response curves for (A) methyl-2-methoxybenzoate, (B) methyl-4-methoxybenzoate, (C) 2-amino-4,5-dimethoxyacetophenone, (D) 2-amino-4,5-dimethoxybenzoic acid, (E) methyl benzoate, and (F) acetophenone. Consumption is scaled relative to pretreatment water consumption. Solid dots depict fluid intake on the day the birds received chemical in their drinking water. Open circles depict relative water intake on the day following treatment. Vertical bars depict 1 SE. Inset: The Duncan's multiple-range post-hoc comparison for fluid intake for concentration groups on the day of treatment. Concentration groups are ranked based on relative fluid consumption, i.e., lowest to highest. Lines depict nonsignificant associations between concentration-group means.

28% of normal, with the overall pattern for relative fluid intake between the treatment and posttreatment periods differing ($F = 4.41$, $df = 5,30$, $P = 0.004$; Figure 2C, 2A45DMAP). The post-hoc test for fluid intake during the treatment period showed that consumption for the 2135 mg/liter and 427 mg/liter groups differed from that of groups receiving 43 mg/liter and below. Fluid intake was constant across time for each of the concentration-groups, indicating that repellency was sensory and not postingestionally conditioned ($P = 0.727$).

Intake of 2-amino-4,5-dimethoxybenzoic acid was not reduced relative to normal consumption. There was no significant interaction between concentration group and treatment period ($P = 0.408$), nor were there significant main effects for concentration or period ($P = 0.074$ and 0.665, respectively), i.e., fluid intake during the treatment and posttreatment periods was the same as for the pretreatment period for all test groups (Figure 2D, 2A45DMBA).

Fluid intake for methyl benzoate was reduced to only 63% of normal levels (Figure 2E, MB). Relative fluid intake differed for the treatment and posttreatment periods as a function of concentration ($F = 2.70$, $df = 5,30$, $P = 0.04$). The post-hoc test indicated that only the highest concentration tested (4335 mg/liter) differed from any of the lower concentrations tested. Posttreatment consumption was uniformly similar to pretreatment levels, suggesting that intake of methyl benzoate did not have any carryover effects. Neither was there evidence of a learned avoidance (bihourly) response during treatment administration ($P = 0.719$). Thus, the weak repellent effect was construed to be sensory in nature.

Maximal reduction of fluid intake for acetophenone was 34% of normal consumption (Figure 2F, AP). Relative intake for the treatment and posttreatment periods differed as a function of concentration ($F = 5.0$, $df = 5,30$, $P = 0.002$). The post-hoc comparison of relative fluid consumption during the treatment period indicated that intake for the 5000, 1000, and 500 mg/liter groups were similar, but differed from intake recorded for the 100, 50,10 mg/liter concentration groups. There was no evidence of a postingestional conditioned avoidance (i.e., absolute intake across time for all concentration groups was similar, $P = 0.465$).

Fluid intake for sodium benzoate was equal to normal (Figure 3A, SB). Relative intake profiles for the treatment and posttreatment periods were similar ($P = 0.119$). Although the dose–response curve for the treatment period indicated that there was no repellent effect, the significant period effect ($F = 29.93$, $df = 2,60$, $P \leq 0.001$) indicated that SB exerted a carryover effect. The increased loading experienced by birds in the higher concentration groups resulted in increased water consumption (higher than normal) during the posttreatment period. While there was no evidence of a sensorially mediated repellent effect, there was some evidence of a postingestional effect. The average concentration profiles across time were all similar ($P = 0.065$). However, there was a tendency

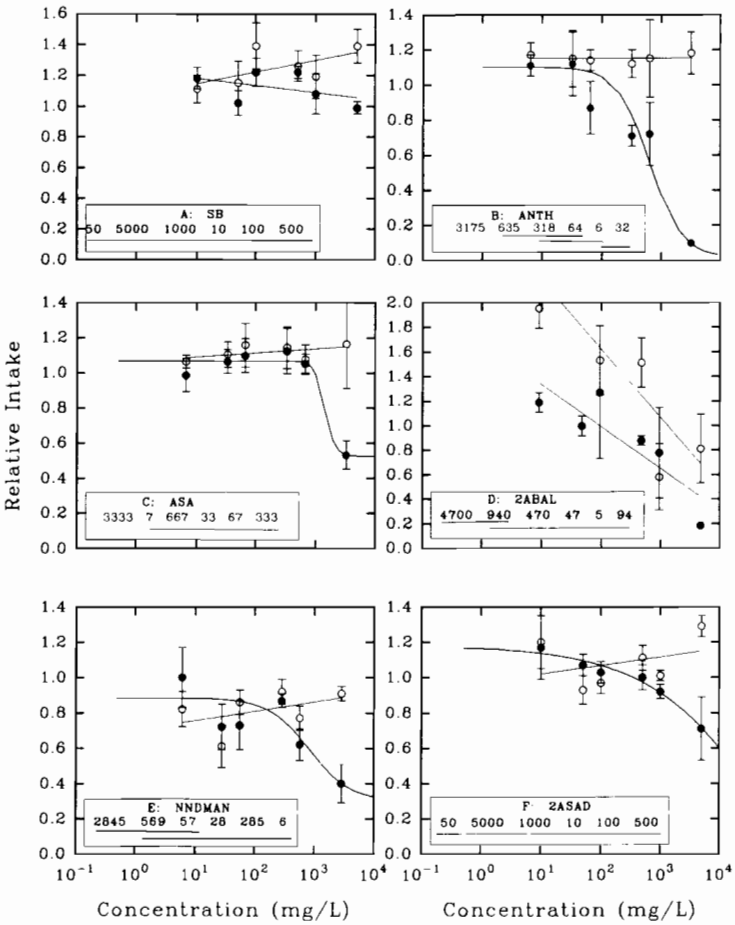


FIG. 3. Dose-response curves for (A) sodium benzoate, (B) anthranil, (C) acetylsalicylic acid, (D) 2-aminobenzyl alcohol, (E) *N,N*-dimethylanaline, and (F) 2-aminobenzene sulfonic acid. Refer to Figure 2 for description of figure particulars. Note the change of scale for intake for parts A and D.

for birds to drink less treated fluid as a function of time ($F = 28.93$, $df = 2,60$; $P < 0.001$). Thus, prolonged exposure (i.e., >4–6 hr) to SB might yield repellency in the context of a classical conditioned-avoidance paradigm.

Anthranil maximally suppressed fluid intake to 10% of normal consumption (Figure 3B, ATHN). The relative intake profiles for the treatment and posttreatment period were different ($F = 11.96$, $df = 5,30$, $P < 0.001$). The post-hoc test showed that intake for birds presented with 3175 mg/liter was different from

phenone) did not substantially change the birds' sensitivity relative to the OAP reference, but it did result in a decrease in suppressive activity (Figure 6C).

Removing the EDG from MA (e.g., methyl benzoate) resulted in a decrease in suppression, but no substantial shift in sensitivity (Figure 6A). In contrast, there was a loss of sensitivity and suppressive activity for acetophenone (relative to OAP, Figure 6C). The EWG does not by itself detract from repellency. Anthranil possessed approximately the same level of sensitivity and suppression as MA (Figure 6C), indicating the importance of the acidic function of EWGs in detracting from repellency. The importance of acidity as a repellent detractor is further seen in the poor suppressive activity of 2-aminobenzene sulfonic acid.

Removing the EWG (e.g., *N*,*N*-dimethylaniline) decreased the suppressive activity but enhanced the functional response to concentration relative to MA. However, because *N*,*N*-dimethylanaline still clustered with other repellents, the data suggest that the absence of an EWG is not critical to repellency.

DISCUSSION

In general, our qualitative predictions about repellent activity were borne out by the data. However, the current study revealed several modifications to the general model. The presence or absence of EWGs or EDGs are important contributory factors for repellency, but the presence of both is not necessary for repellency. For example, despite the absence of an EDG and the consequent effect of electron depletion due to the EWG, acetophenone was repellent. However, the degree of suppression and sensitivity were diminished relative to responses observed for OAP (Table 1, Figure 6C). In the case of methyl benzoate, the diminution of suppressive activity relative to MA was sufficiently large so as to render methyl benzoate ineffective as a repellent (Figure 6A). Thus, while not critical for repellency, the EDG does contribute to enhancing repellency. Similarly the absence of an EWG does not eliminate repellency, but it does enhance repellent action. Birds were less sensitive to *N*,*N*-dimethylanaline and the compound's repellent action was less than either of the two reference molecules (Figure 6B).

It appears that the basicity of the EWG is an important feature regulating repellency of a compound. The presence of a carboxyl or other acidic function on the aromatic ring uniformly results in poor repellent action, irrespective of other attributes of the molecule. The detracting effects are amplified if the acidic function is within the EWG.

The presence of a negative charge on the phenyl ring is an additionally important feature for repellency. The salt of benzoic acid (sodium benzoate) tends to increase the delocalization of lone pairs of electrons between the two oxygens and carbon, resulting in a moderately more basic aqueous solution

(relative to the nonrepellent benzoic acid). Nonetheless, sodium benzoate is not repellent. Because the carboxylic carbon is less electropositive, repellency may be diminished. Benzaldehyde, which possesses a carbonyl group (i.e., is very reactive to nucleophilic attack) but lacks EDG substituents, is a potent repellent (Clark, in preparation). In this case, the alpha carbon is very electropositive and is open to nucleophilic attack. Furthermore, the molecule is more basic relative to sodium benzoate and benzoic acid. So long as substitution at the alpha carbon site is with relatively electron-donating groups, such as CH_3 (e.g., acetophenone), the molecule will exhibit some repellent action. The fact that acetophenone is only moderately repellent and benzaldehyde is strongly repellent, suggests that electrophilicity of the alpha carbon is important.

Simply loading the phenyl ring with EDGs may not yield a better repellent. The addition of OCH_3s in the 4- and 5-carbon positions of 2-aminobenzoic acid and 2-aminoacetophenone might mitigate the effects of electron donation by shifting the pi orbitals out of the phenyl ring's plane, thus causing steric inhibition. Another possibility is that the OCH_3s alter the charge distribution around the phenyl ring relative to the unsubstituted moieties.

Finally, the phenyl ring itself is critical to repellency. Neither β-alanine nor its methyl ester were repellent relative to *o*-aminoacetophenone and methyl anthranilate (the only difference between the pairs of molecules being the presence or absence of the aromatic ring).

In summary, the most critical features of an avian repellent (in order of importance) appear to be: (1) the presence of a phenyl ring; (2) the basicity of the molecule in general, and specifically, when an electron withdrawing group is present, the absence of an acidic function within the EWG functionality; and (3) the electronegativity of the phenyl ring. Steric effects and extreme delocalization of lone pairs of electrons (as occurs with meta isomers and aromatics multiply substituted with EDGs) tends to interfere with repellency. These features are typified by two well-described avian repellents, methyl anthranilate and *o*-aminoacetophenone (MA-OAP).

Considerable effort has been directed towards the identification of mammalian chemoreceptor types, but no work has focused on birds. For mammals, numerous models have been put forward to explain perception of chemically induced pain (Nielsen, 1991). These models generally belong to one of three categories. First, stimuli, e.g., acids, may cause physical damage to cells, resulting in release of endogenous substances such as bradykinins, serotonin, and histamine, which bind to specific receptor sites on chemoreceptive fibers, resulting in neuronal depolarization. Second, external stimuli may specifically bind to receptors directly. Third, external stimuli may adsorb near the chemoreceptive fiber surface and the interaction of the physicochemical effects may nonspecifically cause neuronal depolarization. It is important to bear in mind that the mechanism for sensory irritation need not be mutually exclusive. There is ample

evidence to suggest that all three types of mechanisms exist within trigeminal/somatosensory chemoreceptive fibers.

The form of a mammalian capsaicin receptor may consist primarily of a benzene site associated with a thiol/hydrogen bond donating site that reacts with the C—C double bond and hydrogen bond acceptor site (Solzcsanyi and Jancso-Gabor, 1975; Nielsen, 1991). Interaction with the thiol site is assumed to be critical for the activation of the benzene site. Thus, the long-chain alkyl and the aromatic OCH_3 and OH are all critical features of the mammalian capsaicin receptor. Capsaicin and its analogs do not influence avian irritation. However, vanillyl derivatives lacking the long alkyl chain do act as avian irritants (Shah et al., 1991). This suggests that the thiol site is absent in birds and in its place may be a charged protein. This protein would interact with the EDG, either as a hydrogen donor or via electrostatic attraction. Thus, the difference between the mammalian capsaicin receptor and the proposed avian methyl anthranilate/*o*-aminoacetophenone receptor may simply reflect the loss of expression of a thiol site. While this model clearly requires careful experimental consideration, it does suggest that a broader comparative approach toward vertebrate irritants is warranted.

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